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TITLE: Total Synthesis of Eleutherobin and Analogs and Study of Anti-Cancer Mechanism

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CONTRACTING ORGANIZATION: Sloan-Kettering Institute for Cancer Research
New York, New York 10021

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13. ABSTRACT *(Maximum 200 Words)*

Eleutherobin is a potent anti-cancer natural product. Progress on an improved eleutherobin synthesis is reported herein. Since PI has completed the postdoctoral training, this should be considered as the final progress report.

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Table of Contents

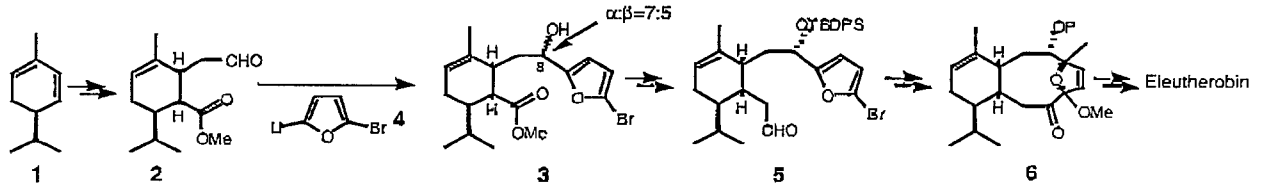
Introduction:

The proposed research for this grant was the total synthesis of eleutherobin. Significant results has been achieved towards an improved synthesis of eleutherobin, which is summarized as Part I in the report. On the other hand, at that time several efficient eleutherobin syntheses had already been accomplished, including one from Danishefsky group. Thus it was decided that the focus of the research was shifted to the synthesis of a pair of potent anti-tumor compounds, the CP compounds, which are synthetically more challenging. The work on CP is summarized in Part II.

PART I Second-Generation Eleutherobin Synthesis

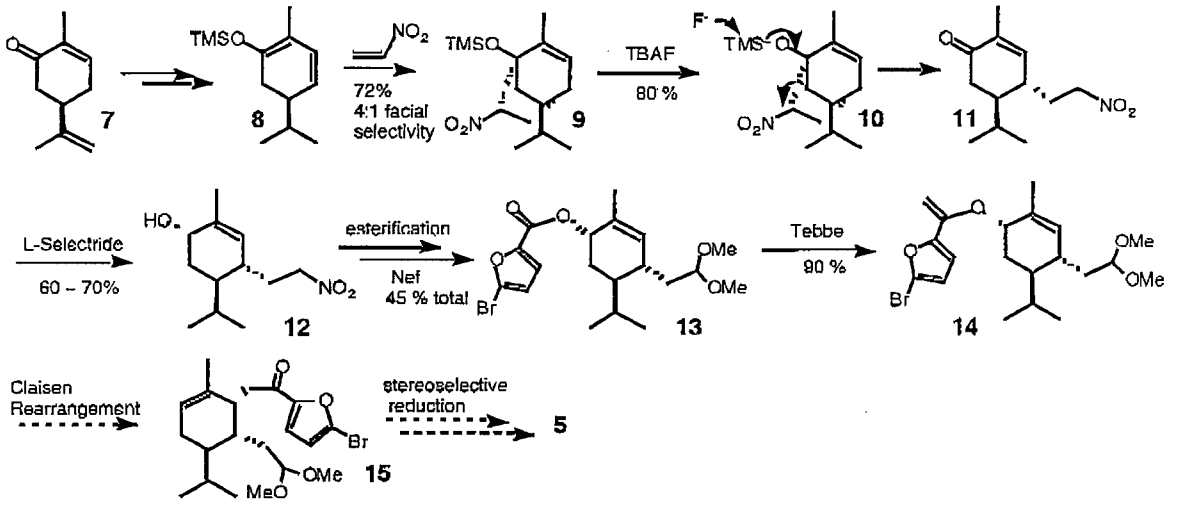
Eleutherobin was first isolated from the soft coral *Eleutherobia sp.* as a novel marine natural product. It has a similar tumor-type selectivity mimicking Taxol, one of the best breast cancer treatment. Moreover, eleutherobin has its own advantage that it is more water soluble and more effective against some multiple drug resistant or Taxol-resistant cell lines. On the other hand, eleutherobin has a very limited supply from the natural sources, thus an efficient total synthesis is very important for producing large quantities of eleutherobin and its analogs for testing. Although the total synthesis of eleutherobin had been accomplished in several labs including Danishefsky group, we felt that the synthesis still could be improved, as stated below.

Scheme 1



The above Scheme 1 outlines the initial eleutherobin synthesis developed in this lab (ref.1). It features addition of 2-bromo-5-lithio furan **4** to aldehyde **2** to give the key intermediate **5**. The C8 epimer is also isolated as a substantial side product (ca. 7:5 diastereomeric ratio). Although the undesired epimer can be recycled, the efficiency of the synthesis certainly has a room for improvement. Moreover, we would like to directly install a two carbon piece to avoid the steppy homolongation of the one carbon piece (from **3** to **5**).

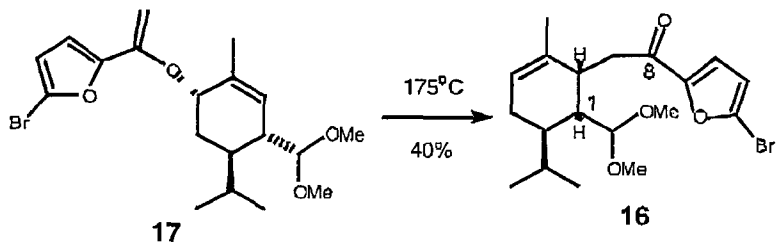
Scheme 2



Scheme 2 indicates the recent progress on this front, which was achieved since the last progress report. It outlined our modified synthesis toward the intermediate **5**, starting from known compound **8** (from **7**, see ref. 2). For the start, a regio- and stereo-selective Diels-Alder reaction gave **9** as the major product. Driven by the release of the ring-strain, deprotection of TMS leads to a Retro-Henry type fragmentation to give the two-carbon side chain.

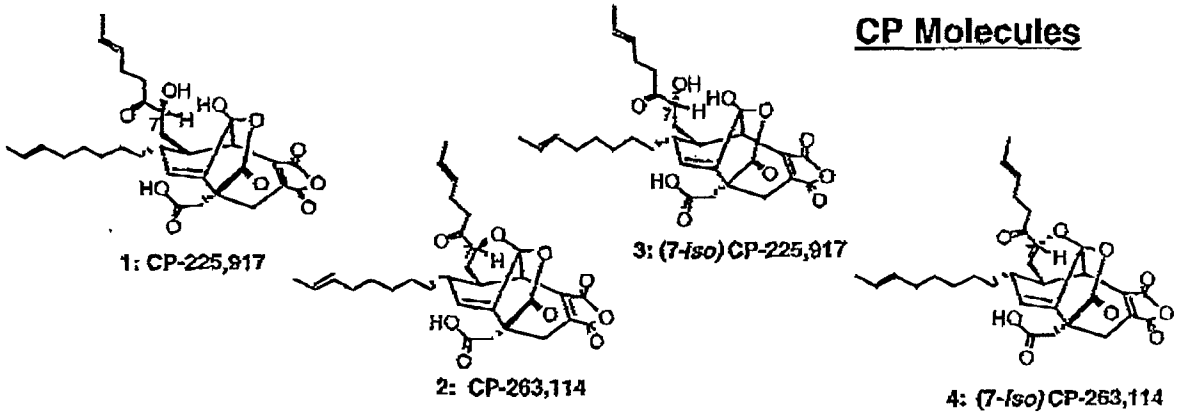
Next, we wish to install the furan piece through a Claisen rearrangement of allylic vinyl ether **14**. After a stereoselective reduction as the result of the equatorial attack of L-selectride to give **12**, esterification, Nef reaction which directly transforms the nitro group to protected aldehyde, and Tebbe reaction gave the Claisen precursor.

During the last progress report, a synthesis toward the intermediate **17** was described so was the conversion of **17** to **18** via the Claisen rearrangement. We hope that by the same token, conversion of **14** to **15**, then **5**, could be achieved.



17 **16**

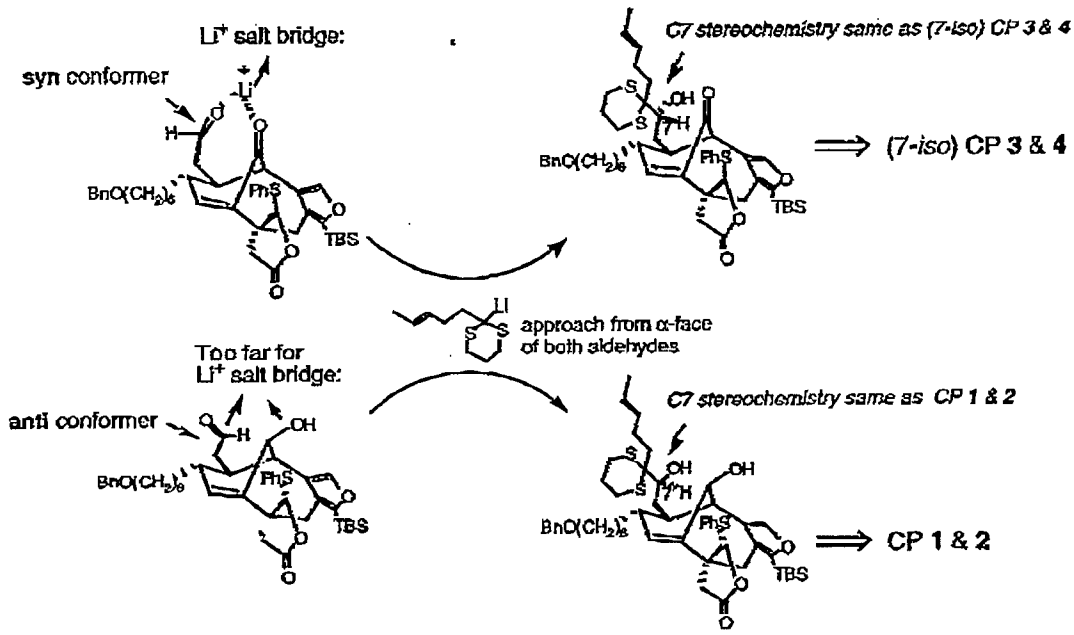
PART II. Total Synthesis of CP Molecules



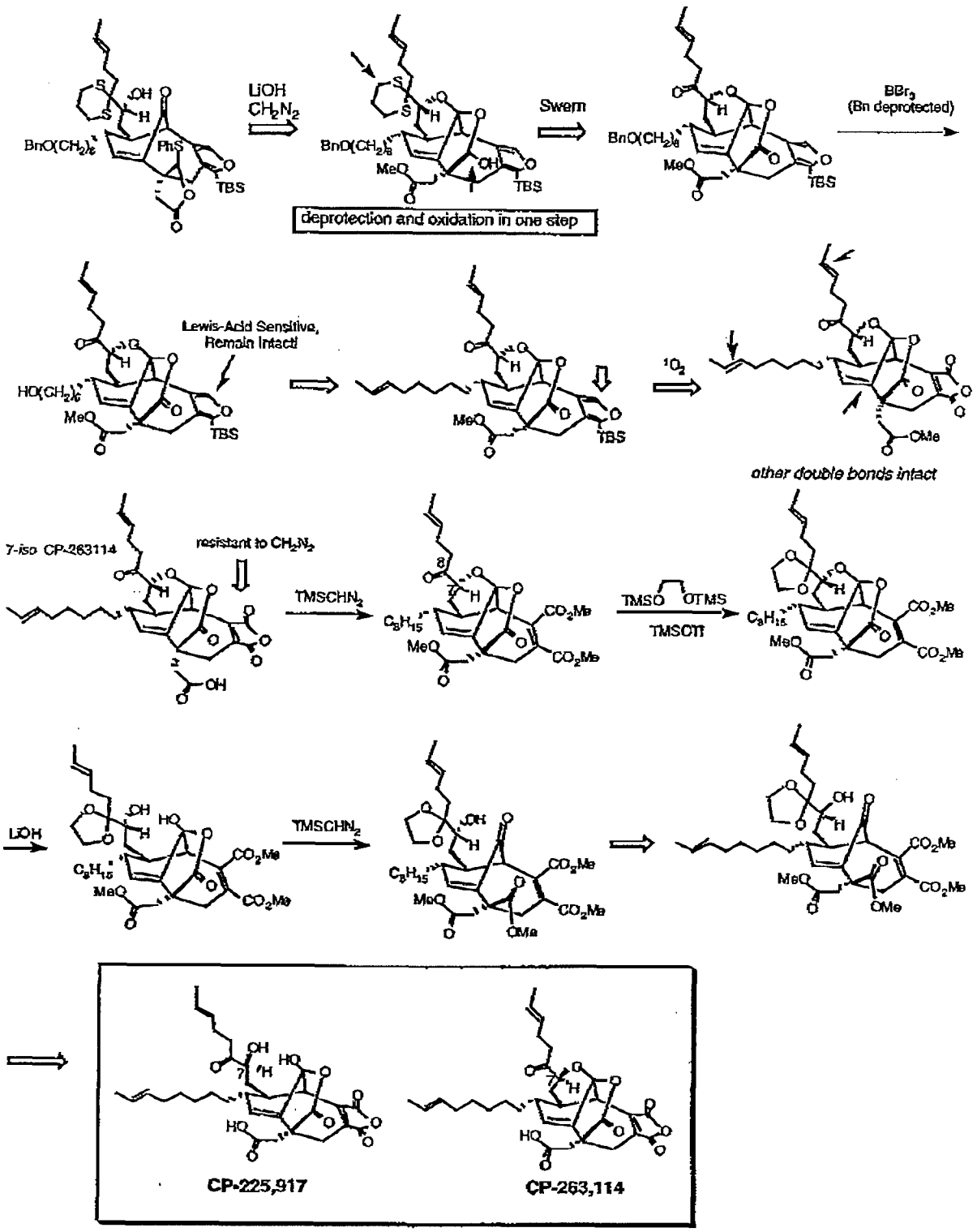
CP compounds 1 and 2 were discovered by Pfizer from the fermentation broth of an unidentified fungus as inhibitors for farnesyl transferase and squalene synthase. Since farnesyl transferase is involved in the functioning of oncogene Ras, these molecules are potential anti-tumor agents.

Farnesyl transferase has been identified as responsible for the farnesylation of ras protein, a process that occurs in the early stages of cell division. When a ras gene is mutated, the molecular switch for cell proliferation may be left in the "on' position, resulting in uncontrolled cell growth (i.e. cancer). In principle, inhibition of ras farnesyl transferase, and thus inhibition of the farnesylation could effectively turn this switch "off", allowing for specific control of cell growth through chemotherapeutic process.

CP-263,114 and CP-225,917 inhibit Ras farnesyl transferase from rat brains with IC_{50} values of 20μM and 6μM, respectively. CP-263,114 inhibits SQS isolated from rat liver microsomes with an IC_{50} value of 160 μM and CP-225,917 with an IC_{50} of 43μM. Hence, molecules of this type appear to be reasonable candidates for the advancement of anticancer medications. With an unusual polycyclic ring system, a densely packed array of oxygen functionalities, quarternary center, an anti-Bredt bridgehead double bond, novel lactone-ketal arrangement and a rare maleic anhydride moiety, the CP molecules are synthetically challenging.



We had achieved the global structure of CP compounds (cf. Dongfang Meng, DAMD17-97-1-7146), but the steric center at C7 was opposite to the natural products. During the past grant period, as the following schema show, we have successfully achieved the installation of the correct C7 and ultimately the synthesis of CP 1 and 2. We were able to use chelation control from a remote group to affect the outcome of a lithio dithiane-aldehyde coupling to achieve different stereo chemistry at C7, for the CP or 7-iso CP series. Moreover, the 7-iso CP compound was converted to the CP compounds via the inversion of C7 hydroxyl through an oxidation-reduction strategy. Treatment of 7-*iso*-CP-263114 or its methyl ester with TMS-diazomethane gave rise to trimethyl ester. The side chain ketone was then protected as a dioxolane using the Noyori method. Opening of the γ-lactone with LiOH, followed by treatment with TMS-diazomethane afforded ester. Dess-Martin oxidation furnished the diketone. Treatment with $LiAlH(OtBu)_3$ was able to selectively reduce the C7 ketone without affecting the C1 ketone to give a 1:1 C7 diastereoisomers at C7. We attribute the regio- and equal face selectivity of the reduction to the assitance of the neighboring dioxolane as it may complex with the reducing reagent to both faces of the C7 ketone. For instance, the keto dithiane analog undergoes more rapid reduction at the C1 ketone relative to that at C7. Finally, all four methyl esters and the dioxolane protection were removed by hydrolysis under LiOH and TFA, respectively, to furnish the CP-225917 (**2**), which can be converted to CP-263114 (**1**) upon treatment with MSA.


LiOH
CH_2N_2
Swern
BBr_3
(Bn deprotected)
deprotection and oxidation in one step
Lewis-Acid Sensitive,
Remain Intact!
$^1O_2$
other double bonds intact
7-iso CP-263114
resistant to CH_2N_2
$TMSCHN_2$
TMSO OTMS
TMSOTf
LiOH
$TMSCHN_2$
CP-225,917
CP-263,114

Key Research Accomplishments:

1. Critical steps have been established on an improved eleutherobin synthesis. The rest of the strategy, i.e., Claisen rearrangement, has been validated on the model system.
2. Total synthesis of the novel anti-tumor CP compounds have been achieved.

Reportable Outcomes:

Publications:

1. The Synthesis of CP-263,114 and CP-225,917: Striking Long-Range Stereocontrol in the Fashioning of C7

Qiang Tan, Samuel J. Danishefsky

Angewandte Chemie International Edition

Volume: 39, Issue: 24, Pages: 4509-4511

2. Discovery Through Total Synthesis - Epimerization at C7 in the CP Compounds: Is (7S)-CP-263,114 a Fermentation Product?

Dongfang Meng, Qiang Tan, Samuel J. Danishefsky

Angewandte Chemie International Edition

Volume: 38, Issue: 21, Pages: 3197-3201

Conclusions:

Key steps toward an improved eleutherobin synthesis have been completed. This is an important step toward seting the stage for the large-scale synthesis of Eleutherobin and its analogs. On the CP project, the completion of the synthetic CP compounds not only gives an alternative method to achieve those potent anti-tumor agents, and open the gate for structural modifications, but also demonstrate the power of the modern organic synthetic techniques in making such complex structures.

Conclusions:

Key steps toward an improved eleutherobin synthesis have been completed. This is an important step toward seting the stage for the large-scale synthesis of Eleutherobin and its analogs. On the CP project, the completion of the synthetic CP compounds not only gives an alternative method to achieve those potent anti-tumor agents, and open the gate for structural modifications, but also demonstrate the power of the modern organic synthetic techniques in making such complex structures.

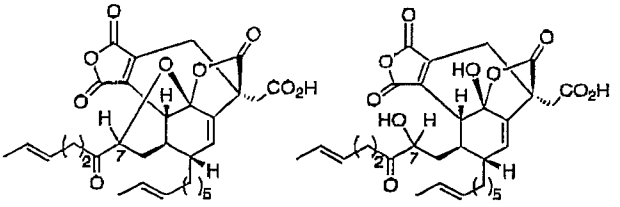
Appendices:

Attached are PI 's publications during the grant period.

The Synthesis of CP-263,114 and CP-225,917: Striking Long-Range Stereocontrol in the Fashioning of C7**

Qiang Tan and Samuel J. Danishefsky*

During a screening exercise which was designed to target inhibitors of Ras farnesyl transferase and squalene synthase, Pfizer scientists reported the isolation of two natural products CP-263,114 (**1**) and CP-225,917 (**2**) as fungal metabolites extracted from juniper twigs in Texas.[1] These compounds have fostered a great deal of creative research from synthetic organic chemists. The interest accrues from the novel and challenging structures of the CP metabolites rather than from compelling biological imperatives. Three total syntheses of **1** and **2** have been described.[2] The first of these was reported by Nicolaou and co-workers.[2a-c] When appropriately modified, their synthesis revealed the configuration of **1** and **2**. These compounds are now known to correspond to the absolute stereostructures shown. This finding was independently confirmed by the groups of Shair[2d] and Fukuyama.[2e]



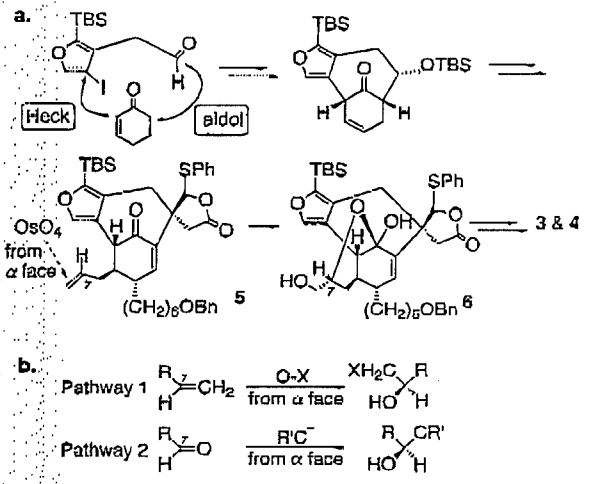
1: 7-H= α (CP-263,114)
3: 7-H= β (7-*epi*-CP-263,114)

2: 7-H= α (CP-225, 917)
4: 7-H= β (7-*epi*-CP-225, 917)

In our earlier report[3] we disclosed the total synthesis of the 7-*epi* series of the CP compounds (**3** and **4**). We are now confident that these 7-*epi* compounds are themselves less abundant natural products, found in the fermentation broth.[4] Nonetheless, since the goal structures of our synthetic venture were compounds **1** and **2**, we undertook the challenge of reaching these natural products. In the course of this study, we encountered some remarkable instances of stereochemical guidance by remote functional groups arising from the novel architectures of the pre-CP intermediates. These findings, as well as the attainment of our synthesis goals in reaching **1** and **2**, are described herein.

On casual inspection it would appear that the synthetic problem could readily be solved by equilibration of **3** or **4**, or their precursors, at C7. However, as was detailed in our total synthesis report,[3d] epimerization at C7 under apparent thermodynamic control only proceeds in the direction of the 7-*epi* diastereomer. Hoped for solutions based on kinetic quenching of enolates derived from deprotonation at C7 under irreversible conditions were unsuccessful. Complicating both of these strategies for inverting the C7 configuration was the general instability of the CP systems to several intended deprotonation protocols. Hence it was necessary to retreat to earlier stages of the synthesis to accomplish our objective of reaching **1** and **2** through our total synthesis.

The reaction that established the eventual 7-*epi* stereochemistry arose from the action of osmium tetroxide on the side-chain allyl group of compound **5** (Scheme 1a). This oxidation resulted in a hemiacetal bearing a hydroxymethyl



Scheme 1. TBS = *tert*-butyldimethylsilyl, Bn = benzyl.

group at C7. The dihydroxylation was essentially stereospecific. The hemiacetal was shown to have the stereochemistry of **6** by its eventual conversion into **3** and **4**. In retrospect this result can be explained by α-face attack of the oxidant upon an "extended *anti*" conformation as proposed for **5** (Scheme 1a). While this was a disappointing result at the time, a seemingly workable solution virtually suggested itself. The thought was to gain access to the required C7 side-chain stereochemistry by inverting the order of element linkage to the achiral C7 sp^2 precursor (Scheme 1b). Thus, in the dihydroxylation reaction (Pathway 1) an oxygen atom had been added to the C7 methane carbon of a terminal methylene group. Now we hoped to reverse the stereochemical outcome by adding a carbanion equivalent to a C=O linkage (Pathway 2).

Specifically we sought to add lithio dithiane **8** to aldehyde **10**. Of course the successful realization of the scheme

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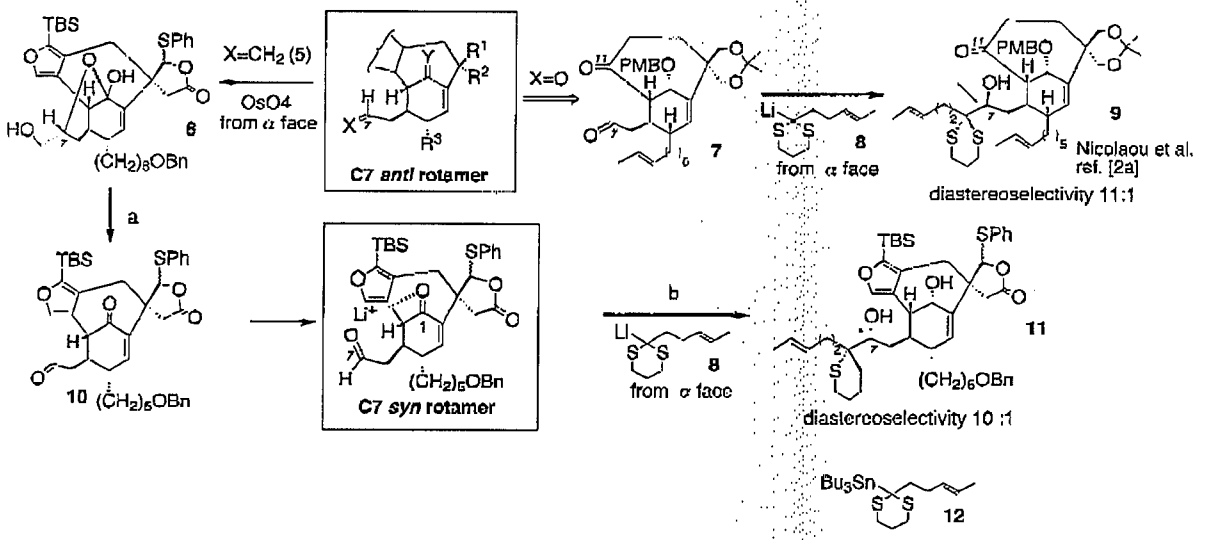
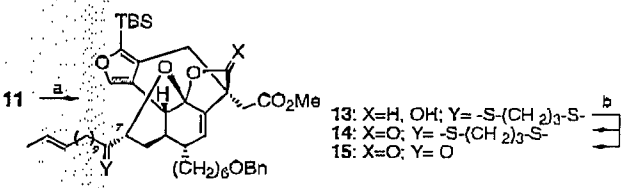


Scheme 2. a) $Pb(OAc)_4$, PhMe, 0 °C, 20–45 min, 90 %; b) **12**, MeLi/LiBr, THF, −78 °C, then add aldehyde at −200 °C, warm to −78 °C, 15 min, 70 % total. PMB = *para*-methoxybenzyl.

presumed that the conformation of the reacting formyl group of **10** would also be "extended *anti*" and that the nucleophile would again (as with the osmium tetroxide reaction) attack from the α-face. Indeed as we were preparing for this very experiment, Nicolaou and co-workers reported that the addition of lithio dithiane **8** to aldehyde **7** produced **9** in an approximately 11:1 ratio relative to the C7 epimer (Scheme 2).[2a] The result from the Nicolaou group was consistent with our dihydroxylation result in that opposite diastereomers had been produced at C7 following the opposite linkage orders.

Notwithstanding its dominantly hemiacetal character, **6** reacted with lead tetraacetate to afford **10** (Scheme 2). The latter reacted smoothly with **8** (generated in situ from its *n*-butylstannyl derivative **12**) to afford substantially a single carbinol in a 10:1 ratio with the C7 diastereomer. Our satisfaction with the result was short-lived when it was learned that the carbinol was **11**. This realization followed a two step conversion of **11** into **13** followed by oxidation of the γ-lactol and the deprotection of the dithioketal (Scheme 3). The resultant **15** had been previously encountered in our synthesis of the 7-*epi* systems **3** and **4**.

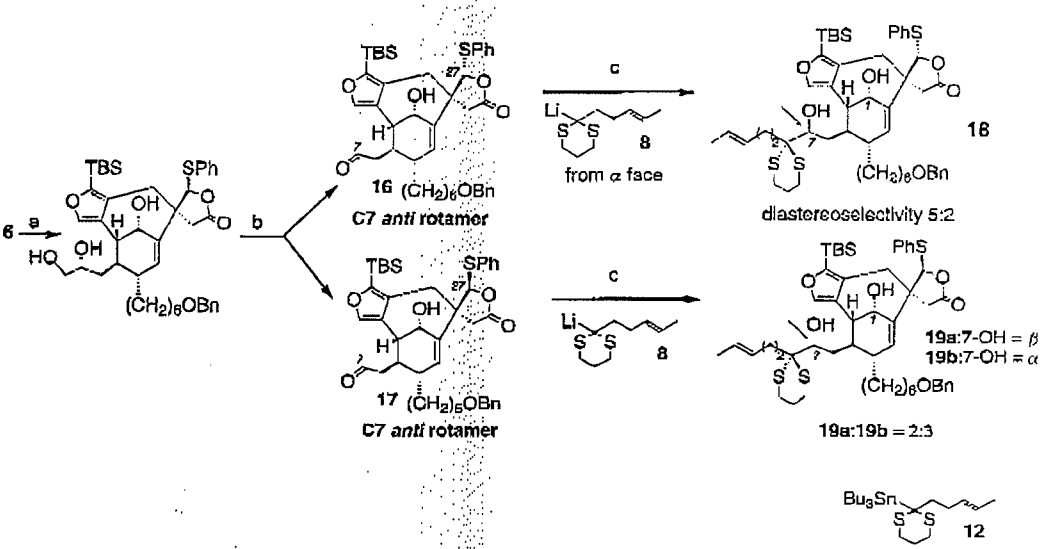
It seemed that a factor to explain the massive difference (11:1 versus 1:10) between our case and that of Nicolaou and co-workers was the absence or existence of the C1 ketone. Perhaps the presence of this ketone in our substrate **10** occasioned a shift in either



Scheme 3. a) 1. LiOH, THF, 36 h; 2. CH_2N_2, 60 %; b) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, −78 °C, 75 %, **14**:**15** = 1:1.

the nature of the reactive aldehyde rotamer or the sense of attack on the corresponding rotamer. For instance, formation of a local "lithio channel", by the C1 ketone and the C7 aldehyde, would favor an otherwise unfavorable *syn*-like rotamer as shown in Scheme 2.

This argument was evaluated following conversion of **6** into **16** and **17** as shown in Scheme 4. While not productive with respect to our goal, these studies provided striking instances



Scheme 4. a) $NaBH_4$, PhMe/*i*PrOH, 6.5 h, 70 %; b) $Pb(OAc)_4$, PhMe, 0 °C, 20–45 min, 90 %; c) **12**, MeLi/LiBr, THF, −78 °C, then add aldehyde at −200 °C, warm to −78 °C, 15 min, 70 % total.

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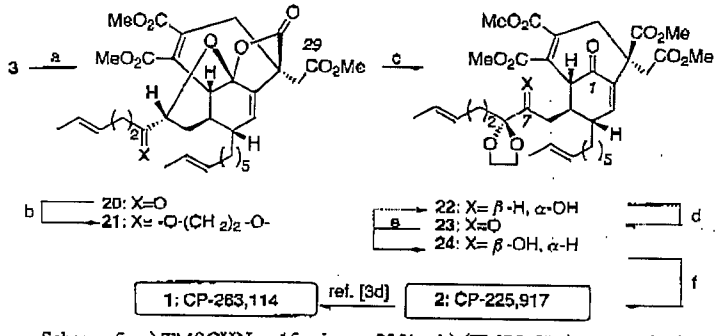
of long-range effects. Indeed, even the two C27 epimeric thiophenyl compounds reacted quite differently with **8**. In the case of **16**, the ratio of **18** and its C7 epimer was 5:2, whereas with **17**, the ratio of **19a**:**19b** was 2:3.[5]

Qualitatively at least, removal of the C1 ketone did markedly shift the sense of addition of anion **8** in the predicted sense. Failure to achieve the very high selectivity described by the Nicolaou and co-workers[2a] may be a consequence of the presence of the C11 ketone in their substrate **7**. This ketone could well provide additional guidance for α-face attack by the nucleophile on the rotamer shown.

While **18** and **19a** are potentially valuable precursors toward the CP systems **1** and **2**, a more rapid progress was registered. This involved the reduction of a C7 ketone to reach our goal (Scheme 5). Here too neighboring group influences



Scheme 5. a) $TMSCHN_2$, 15 min, >90%; b) $(TMSOCH_2)_2$, TMSOTf, CH_2Cl_2, 5 h, 90%;[6] c) 1. LiOH, THF/H_2O, 1.2 h; 2. $TMSCHN_2$, 0.5 h, 70% overall; d) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 4 h, 90%; e) $LiAlH(OtBu)_3$, toluene, −10°C, 5 min, 70%, **22**:**24** = 1:1; f) 1. LiOH, $THF-H_2O$, 24 h; 2. $TFA/CH_2Cl_2/H_2O$ (54/4/1), 30 min, 70% overall.

are pivotal. The sequence started with the interesting reaction of trimethylsilyldiazomethane with **3** to afford **20** and thence **21**. The latter reacted with lithium hydroxide and then trimethylsilyldiazomethane to give **22**, which following oxidation yielded **23**. Treatment of this compound with lithium tri(*tert*-butoxide) hydride provided **22** and **24**. We note again[3d] that, in the case of **21**, a "cascade"[2b] driven by carboxylate participation can not be invoked, since the lactone saponification occurs with the C29 methylester intact.

It is also likely that the regiospecific reduction of the C7 ketone relative to that at C1 is orchestrated by the proximal dioxolane protecting group. For instance, the corresponding ketodithiane analogue undergoes more rapid reduction at the C1 ketone relative to that at C7. The reduction of **23** as shown affords **24** as well as the separable and recyclable **22**. The four methyl esters are cleaved through long-term treatment of **24** with lithium hydroxide and reconstruction of the system was accomplished through the action of TFA (see structure **2**). The conversion of **2** into **1** has been reported.[3d]

The contrathermodynamic conversion of the **3**, **4** minor series of metabolites into the more prevalent **1**, **2** family has been accomplished, which thus completes our file on all the known components of the fermentation mixture. Clearly the densely funtionalized architectures of these four compounds and their synthetic precursors give rise to quite striking intramolecular signaling which invites further experimentation and elucidation.

Received: August 22, 2000 [Z15679]

[1] a) T. T. Dabrah, T. Kaneko, W. Massefski, Jr., E. B. Whipple, *J. Am. Chem. Soc.* **1997**, *119*, 1594; b) T. T. Dabrah, H. J. Harwood, Jr., L. H. Huang, N. D. Jankovich, T. Kaneko, J.-C. Li, S. Lindsey, P. M. Moshier, T. A. Subashi, M. Therrien, P. C. Watts, *J. Antibiot.* **1997**, *50*, 1.

[2] For total syntheses, see: a) K. C. Nicolaou, P. S. Baran, Y.-L. Zhong, H.-S. Choi, W. H. Yoon, Y. He, K. C. Fong, *Angew. Chem.* **1999**, *111*, 1774; *Angew. Chem. Int. Ed.* **1999**, *38*, 1669; b) K. C. Nicolaou, P. S. Baran, Y.-L. Zhong, K. C. Fong, Y. He, W. H. Yoon, H.-S. Choi, *Angew. Chem.* **1999**, *111*, 1781; *Angew. Chem. Int. Ed.* **1999**, *38*, 1676; c) K. C. Nicolaou, J. K. Jung, W. H. Yoon, Y. He, Y.-L. Zhong, P. S. Baran, *Angew. Chem.* **2000**, *112*, 1899; *Angew. Chem. Int. Ed.* **2000**, *39*, 1829; d) C. Chen, M. E. Layton, S. M. Sheehan, M. D. Shair, *J. Am. Chem. Soc.* **2000**, *122*, 7424; e) N. Waizumi, T. Itoh, T. Fukuyama, *J. Am. Chem. Soc.* **2000**, *122*, 7825. For synthetic approaches, see: f) K. C. Nicolaou, M. W. Harter, L. Boulton, B. Jandeleit, *Angew. Chem.* **1997**, *109*, 1243; *Angew. Chem. Int. Ed.* **1997**, *36*, 1194; g) K. C. Nicolaou, M. H. D. Postema, N. D. Miller, G. Yang, *Angew. Chem.* **1997**, *109*, 2922; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2821; h) H. M. L. Davies, R. Calvo, G. Ahmed, *Tetrahedron Lett.* **1997**, *38*, 1737; i) P. W. M. Sgarbi, D. L. J. Clive, *Chem. Commun.* **1997**, 2157; j) A. Armstrong, T. J. Critchley, A. A. Mortlock, *Synlett* **1998**, 552; k) N. I. Waizumi, T. Ito, T. Fukuyama, *Tetrahedron Lett.* **1998**, *39*, 6015; l) C. Chen, M. E. Layton, M. D. Shair, *J. Am. Chem. Soc.* **1998**, *120*, 10784; m) M. M. Bio, J. L. Leighton, *J. Am. Chem. Soc.* **1999**, *121*, 890; n) K. C. Nicolaou, P. S. Baran, R. Jautelat, Y. He, K. C. Fong, H.-S. Choi, W. H. Yoon, Y.-L. Zhong, *Angew. Chem.* **1999**, *111*, 532; *Angew. Chem. Int. Ed.* **1999**, *38*, 549; o) T. Yoshimitsu, M. Yanagiya, H. Nagaoka, *Tetrahedron Lett.* **1999**, *40*, 5215; p) D. L. J. Clive, S. Y. Sun, X. He, J. Zhang, V. Gagliardini, *Tetrahedron Lett.* **1999**, *40*, 4605; q) G. A. Sulikowski, F. Agnelli, R. M. Corbett, *J Org. Chem.* **2000**, *65*, 337; r) J. T. Starr, E. M. Carreira, *Angew. Chem.* **2000**, *112*, 1473; *Angew. Chem. Int. Ed.* **2000**, *39*, 1415.

[3] a) O. Kwon, D.-S. Su, D. Meng, W. Deng, D. D'Amico, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 1978; *Angew. Chem. Int. Ed.* **1998**, *37*, 1877; b) O. Kwon, D.-S. Su, D. Meng, W. Deng, D. D'Amico, S. J. Danishefsky, *Angew. Chem.* **1998**, *37*, 1981; *Angew. Chem. Int. Ed.* **1998**, *37*, 1880; c) D. Meng, S. J. Danishefsky, *Angew. Chem.* **1999**, *111*, 1582; *Angew. Chem. Int. Ed.* **1999**, *38*, 1485; d) D. Meng, Q. Tan, S. J. Danishefsky, *Angew. Chem.* **1999**, *111*, 3393; *Angew. Chem. Int. Ed.* **1999**, *38*, 3197. Previously we had referred to the 7-*epi* compounds as 7*S* based on the particular antipod arbitrarily selected to describe the synthesis of the racemate. In fact, the structures we employed were antipodal to the natural series, which thus necessitated a reversal of the designation at C7.

[4] These compounds have now been identified directly in the fermentation mixture as will be described in detail.

[5] The ratios were determined by oxidizing the C1 alcohol of the dithiane adducts (PDC, PhMe, approximately 80%), and comparing the NMR spectra of the resultant ketones with those of **11**. PDC = pyridinium dichromate.

[6] T. Tsunoda, M. Suzuki, R. Noyori, *Tetrahedron Lett.* **1978**, *21*, 1357.

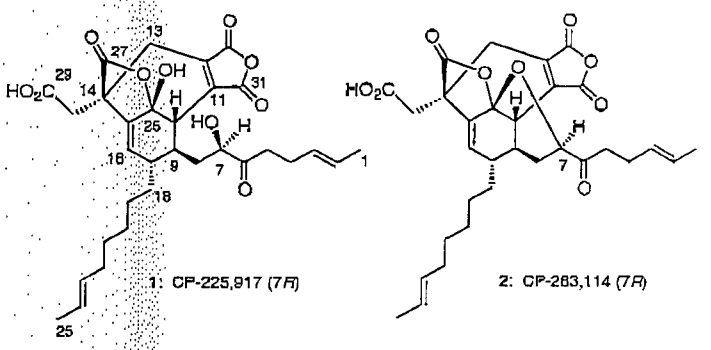
  1433-7851/00/3924-4511 $ 17.50+.50/0

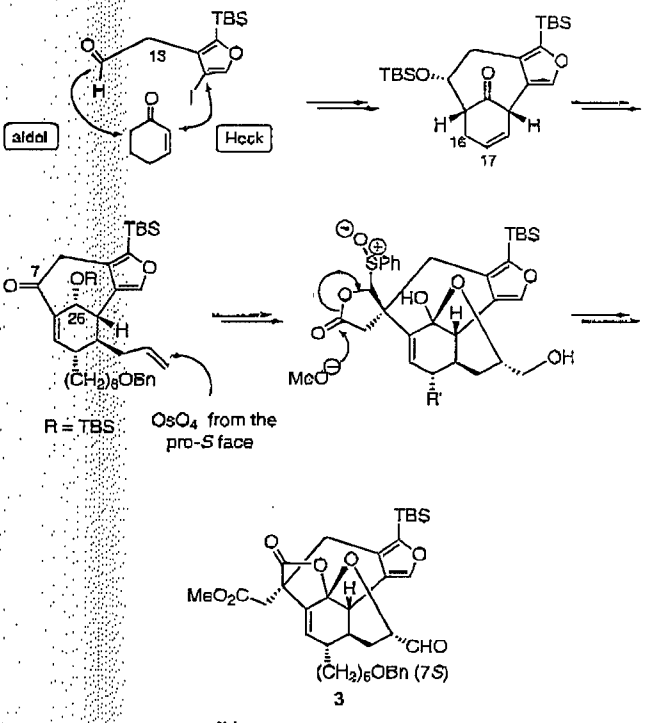
[6] N. Avarvari, N. Maigrot, L. Ricard, F. Mathey, P. Le Floch, *Chem. Eur. J.* **1999**, *5*, 2109–2118.
[7] R. Uson, A. Laguna, in *Organometallic Syntheses, Vol. 3* (Eds.: R. B. King, J. Eisch), Elsevier, Amsterdam, **1986**, pp. 324–325.
[8] X-ray structure determination for **4** ($C_{77}H_{70}AuCl_6GaSi_4P_4$): Crystals of **4** were grown from a solution in CH_2Cl_2; crystal size: 0.28 × 0.24 × 0.15 mm. Data were collected at 123 ± 0.5 K on an Enraf Nonius CAD4 diffractometer using graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). The crystal structure was solved and refined using the Enraf Nonius MOLEN package. Monoclinic, space group *P*21/*c* (no. 14), $a = 12.256(1)$, $b = 23.895(2)$, $c = 26.056(3)$ Å, $\beta = 95.76(2)°$; $V = 7592.1(2.6)$ Å^3; $Z = 4$; $\rho_{calcd} = 1.497$ g cm^{-3}; $\mu = 26.8$ cm^{-1}; $F(000) = 3440$. A total of 15785 unique reflections were recorded in the range $2 \le 2\theta \le 51.8°$, of which 7450 were considered as unobserved ($F^2 < 2\sigma(F^2)$), leaving 8335 for solution and refinement. A non-Poisson weighting scheme was applied with a p factor equal to 0.08. The final agreement factors were $R = 0.049$, $R_w = 0.069$, GOF = 1.35; max./min. residual electron density 2.10(13)/−0.19(13) e Å^{-3}. Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-119267 (**3**) and CCDC-119268 (**4**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
[9] a) C. Elschenbroich, S. Voss, O. Schiemann, A. Lippek, K. Harms, *Organometallics* **1998**, *17*, 4417–4424; b) N. Mézailles, L. Ricard, F. Mathey, P. Le Floch, *Eur. J. Inorg. Chem.* **1999**, *12*, in press.
[10] J. R. Morton, K. F. Preston, *J. Magn. Reson.* **1978**, *30*, 577.
[11] a) A. S. W. Li, L. Kevan, *J. Phys. Chem.* **1981**, *85*, 2557; b) T. Ichikawa, H. Yoshida, A. S. W. Li, L. Kevan, *J. Am. Chem. Soc.* **1984**, *106*, 4324.
[12] A. J. Buck, B. Mile, J. A. Howard, *J. Am. Chem. Soc.* **1983**, *105*, 3381.

Discovery Through Total Synthesis—Epimerization at C7 in the CP Compounds: Is (7*S*)-CP-263,114 a Fermentation Product?**

Dongfang Meng, Qiang Tan, and Samuel J. Danishefsky*

The goal of accomplishing the total syntheses of CP-225,917 (**1**) and CP-263,114 (**2**) has attracted the active participation of a variety of research groups.[1–3] These substances inhibit farnesyltransferase and squalene synthase activity. While the biological potential, if any, of agents that combine both activities is far from demonstrated, chemists have been attracted to this challenge by the novel molecular architecture of these target compounds. Elsewhere, we have described an approach to the synthesis of the CP series that delivered compound **3**, with the full framework to reach the target structure (Scheme 1).[3c]





Scheme 1. Synthesis of **3**.[3c]

We noted that the stereochemistry we were assigning at C7 (*S*)[4] of our synthetic structure was not the same as that assigned by the Pfizer discovery group to CP-263,114 (7*R*).[5, 6] However, one could not then be sure that the assignment to the natural product was necessarily correct. Some preliminary attempts on our part to epimerize aldehyde **3** were not successful and were attended by extensive decomposition. Accordingly, we undertook the installation of the remaining functionality required to go from **3** to the CP compounds (neglecting the issue of the C7 stereochemistry) in the hope of settling this question. Pentenylation of **3** followed by the oxidation of the resultant carbinol afforded **4** (Scheme 2).

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Fortunately, we could deprotect the primary hydroxyl group on the C4 side chain with dichlorodicyanobenzoquinone (DDQ) to afford alcohol **5**. Oxidation of **5** provided aldehyde **6**. The direct coupling of the compound with 1,1-diodoethane[7] gave rise to **7**. NMR spectral analysis continued to suggest that our compounds had the *7S* configuration.[4, 8] At this stage we were in a position to exploit the fused 2-(*tert*-butyldimethylsilyl)furan moiety. Treatment of this compound, as previously described in our model studies[3b] indeed gave rise to the hemiacetal **8** as an anomeric mixture.[9] Oxidation with tetrapropylammonium perruthenate/*N*-methylmorpholine-*N*-oxide (TPAP/NMO) produced the internal carboxylic anhydride **9**.

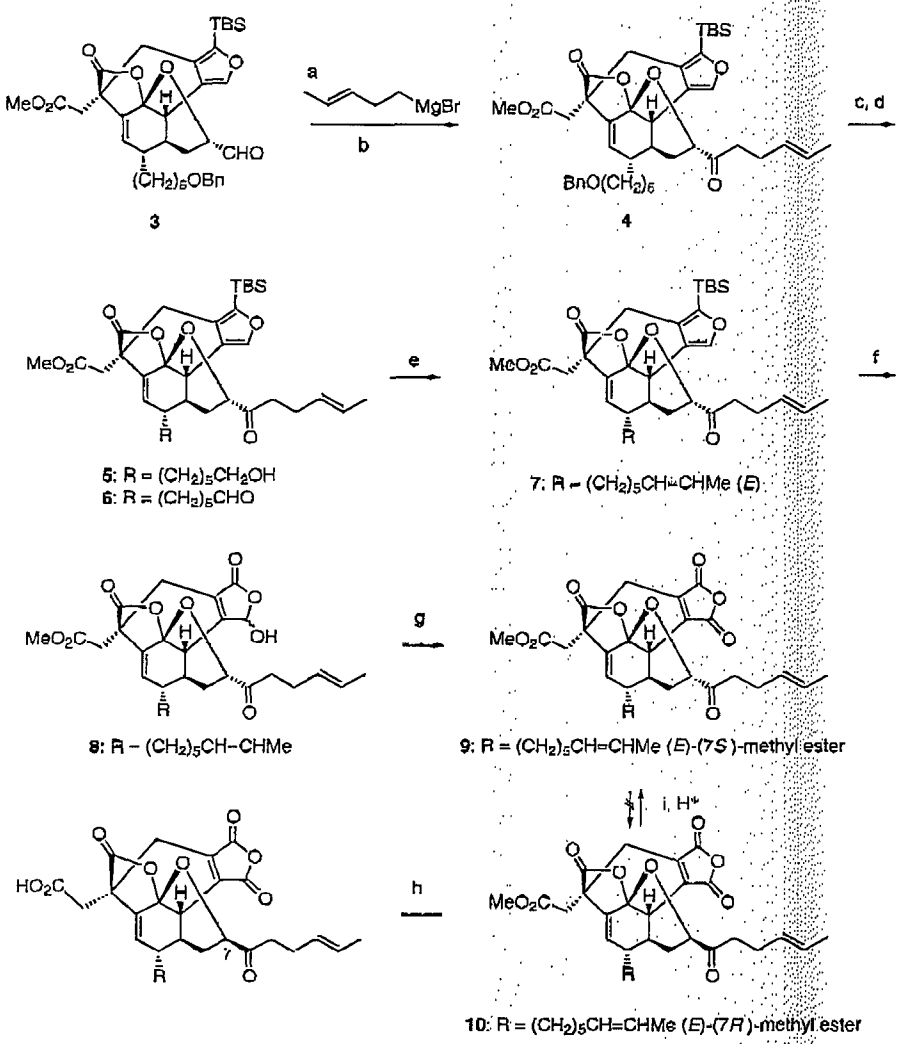
Definitive proof of the configuration of the natural series at C7[4] would require comparison with the methyl ester of CP-263,114, an unknown compound at the time. Of course, the obvious possibility of hydrolyzing **9** to its corresponding acid did not escape our attention. However, in practice, the attempted base-induced saponification of the methyl-ester linkage is superseded by other interesting chemistry (see below).

We were able to convert small reference samples of the acid **2**, made available to us from fermentation,[10] into its methyl ester through the action of diazomethane under carefully controlled conditions.[11] It was clear that the methyl ester **9**[12] obtained from synthesis was similar to, *but not the same as* that derived from **2**. Accordingly, we were obliged to conclude that the natural CP-derived ester indeed corresponds to structure **10**,[13] with the *7R*[4] configuration in accordance with the assignment of Kaneko and colleagues.[5, 6] Correspondingly, the ester derived from total synthesis was, as we had surmised, **9** with *7S* configuration.[3c]

At this point, we recorded a most surprising observation: following esterification of various trace specimens of **2**, provided by Pfizer scientists from various fermentation broths[10]—with **9** used as a reference sample—we could readily detect significant quantities (between 5 and 30%) of the synthetically derived *7S* system **9** in addition to the major product **10**.[14] This finding raised the possibility that the *7S* product may *also* be naturally occurring. We set this question aside and probed whether epimerization at C7 would be possible in the ester series. Interestingly, when a purified sample of **10**, prepared from the methylation of **2** with diazomethane, was subjected to the action of various strong acids such as trifluoroacetic acid (TFA) or, preferably methanesulfonic acid (MSA), there was clear epimerization at C7 leading to a mixture of **9** and **10**. With time, the mixture significantly favored **9**. A precise statement of the ratio is not possible, since some side reactions were occurring as "equilibration" was in progress. We note that after treatment with MSA for one week the ratio **9**:**10** is approximately 3:1. However, equilibrium had not yet been reached.

Unfortunately, attempted equilibration of **9** and **10**, starting with **9**, using TFA or MSA, was attended by serious decomposition in the case of the former acid and essentially no reaction with the latter reagent. These experiments show that the *7S* compound, **9** is substantially more stable than the *7R* compound **10** in the ester series.[4] More extensive investigations of the situation at C7 in the CP-225,917 series are described below.

While the full range of possible acid-catalyzed experiments or other epimerization strategies starting in the manifold of the "closed" 7-*epi*



Scheme 2. a) Diethyl ether, −78°C (80–90% conversion); b) Dess–Martin periodinane, CH_2Cl_2, 60% over two steps; c) DDQ, H_2O, CH_2Cl_2, 60%; d) Dess–Martin periodinane, CH_2Cl_2, 80%; e) 1,1-diiodoethane, $CrCl_2$, THF, 70%; f) *hv*, O_2, rose Bengal, iPr_2EtN, CH_2Cl_2, 0°C; g) TPAP, NMO, CH_2Cl_2, ca. 50% over two steps; h) CH_2N_2, diethyl ether, >90%; i) CF_3COOH or $MeSO_2OH$, $CDCl_3$.

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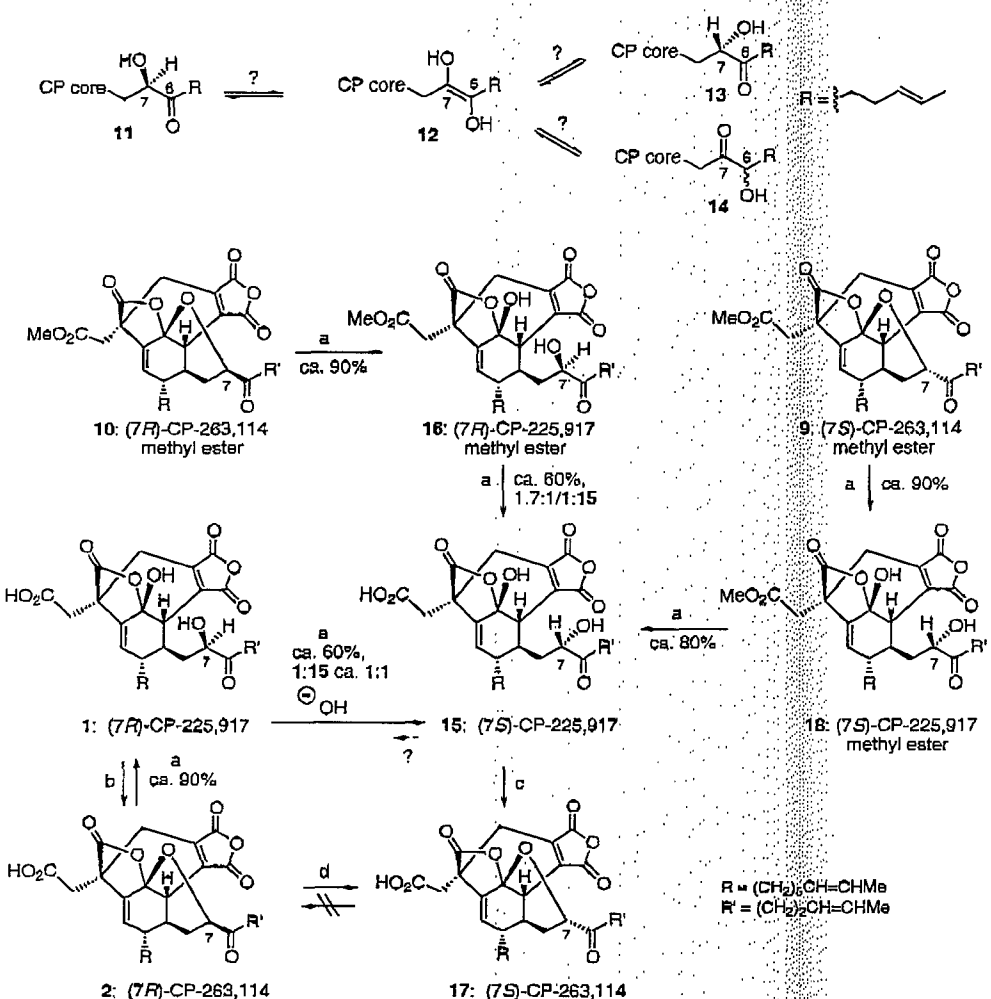
series has not yet been pursued, we posed the question as to whether base-catalyzed epimerization at C7 in the "open" CP series (see structure **1**; where "closed" and "open" refer to the presence and absence, respectively, of an ether bridge between C7 and C28) might be possible (Scheme 3). Such an epimerization could be pictured in terms of the C7-C8 enediols (see partial structure **12**). Clearly, this approach was not without its own attendant risks. In addition to C7 protonation to produce the two stereoisomeric alcohols at C7, there loomed the possibility that ketonization could occur at C7 (partial structure **14**), resulting in a new line of CP congeners that could not readily be "rehabilitated" in our total synthesis venture.

We first probed this question indirectly by starting with a specimen reference sample of the "open" CP acid **1**. In the event, treatment of this compound with lithium hydroxide, generated an approximate 1:1 mixture of **1** and a new acid **15**,[15] which we assumed to be the 7-epimer of **1**. Remarkably, the mixture seemed to be substantially confined to epimers at C7.[16] Thus, starting with **1**, crossing of the C7*R*–C7*S* boundary was possible without significant wandering into the structurally isomeric ketol terrain (→**14**).[16] The lithium hydroxide experiment was also conducted starting with the 7*R* methyl ester **10** of the natural series. The process was closely monitored by HPLC and ^{1}H NMR spectroscopy. The fastest step is that of cleavage of the δ-lactol, which is initiated by a reversible opening of the γ-lactone under formation of the open-chain methyl ester **16**. Concurrently, a slower epimerization at C7 was accompanied by hydrolysis of the methyl ester. After 24 h the ester linkage had been cleaved and the resultant mixture of acids, somewhat richer in **1** relative to **15**,[15] could be separated. Given the fact that the hydroxide-induced conversion of **10**→**16** occurs much more rapidly than hydrolysis of the ester, the critical role postulated by Nicolaou et al. of free carboxylate being a crucial element in a presumed "cascade" process to achieve the opening of the γ-lactone, as judged by cleavage of the δ-lactol, is open to considerable question.[2] In our case, clearly no such participation is involved in the hydroxide-driven opening of the γ-lactone since there are no free carboxylate groups.

Because of some attendant decomposition we cannot quote a precise equilibrium ratio of **1** and **15**. However, we were able to interconnect the open (CP-225,917) and closed (CP-263,114) systems in the 7*S* series by taking advantage of the cyclization reaction with methanesulfonic acid, initially discovered by the Pfizer scientists[6, 7] starting with the natural 7*R* isomer. Compound **1** was indeed converted into **2** exactly as they reported. Similarly, **15** was converted into **17**, the 7*S* analogue of **2**. In each case the cyclization reaction occurred without noticeable epimerization at C7. Long-term treatment of **2** with MSA did result in epimerization at C7. Thus, an 8:1 mixture of **17**:**2** was obtained from **2** after one week.[15, 16] Clearly, the 7*S* acid is substantially more stable than the 7*R* acid **2**, which is in keeping with our findings in the case of the corresponding esters **9** and **10**.

We now had in hand pure samples of the natural (7*R*) "open" (CP-225,917) and "closed" (CP-263,114) series as the acids (**1** and **2**) and the methyl esters (**10** and **16**), as well as the corresponding 7*S* series of closed acid (**17**), closed ester (**9**), open acid (**15**), and open methyl ester (**18**). At this point it was very clear that the reference samples of **2**, obtained from several fermentation runs, con-



Scheme 3. a) LiOH (0.1 M):THF, 1:4; b) $MeSO_2OH$ (1 equiv), $CDCl_3$, ca. 90%; c) $MeSO_2OH$ (3 equiv), $CDCl_3$, ca. 90%; d) $MeSO_2OH$ (15 equiv), $CDCl_3$, ca. 90%, **17**:**2** = 8:1.

tained between 5–30% of **17**. Without an authentic sample such as we had available through total synthesis, it would be quite understandable for the minor 7*S* version of **2** to be overlooked in an isolation program. We also note that the HPLC separation of **2** and its 7*S* epimer is quite difficult.[15]

We then explored the possibility of entering the natural series (7*R*) by base-catalyzed equilibration starting with the 7*S* epimers that could be derived from total synthesis. Remarkably, treatment of **15** with lithium hydroxide followed by acidification gave recovered starting material in addition to some general decomposition. At best, we could detect only trace quantities (about 5%) of **1** by HPLC. However, with the amounts of **15** available to us, fully homogenous CP-225,917 (**1**) was not secured from a total synthesis route.

In summary, the total syntheses of the 7*S*-CP systems has been accomplished. This program, initially directed at the total syntheses of **1** and **2**, has served to broaden our understanding of the chemistry of the CP-225,917 (open) and 263,114 (closed) series and to identify the 7*S* closed isomer **17** in the latter case as a very likely fermentation product. In the closed case a very powerful thermodynamic advantage favoring the 7-epi series (**9**/**10** and **17**/**2**) was discovered. We attribute this striking stability differential to the fact that in the epi series (**9** and **17**) the hexenoyl side chain projecting from C7 is *exo* with respect to the bicyclo-[3.3.1]nonane substructure. By contrast, in the naturally prevalent 7*R* series, the hexenoyl moiety is *endo* and substantially more hindered (Figure 1). A similar conclusion

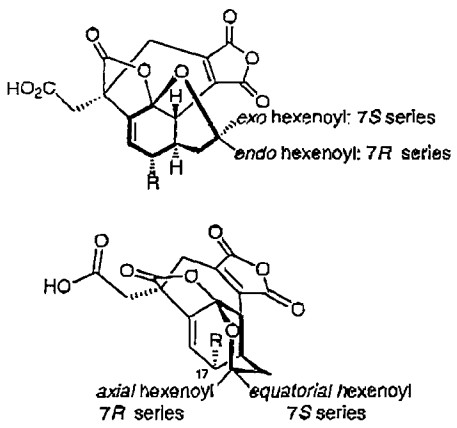


Figure 1. Positioning of the hexenoyl groups in the 7*R* and in the 7*S* series (for further information see the text).

arises from examining the two series from the sterical perspective of the tetrahydropyran ring. If this ring is in a chair conformation, then the hexenoyl group is equatorial in the 7*S* series while it is axial in the 7*R* case (Figure 1). Alternatively, the pyran ring may adapt an energetically costly boatlike conformation in the 7*R* case, to avoid placement of the large hexenoyl group in a 1,3-diaxial relationship to C17. In any case, dynamic equilibration apparently does not lead to detectable conversion of 7*S* into 7*R* diastereomer in the closed systems.

Surprisingly, the preference for the 7*S*-configured system, while perhaps less overwhelming, extends to the open CP-225,917 stereoisomers (**15** and **1**). Here it was initially felt that given free rotation in the open structures, the stability margins between the 7*R* and 7*S* isomers would have been markedly reduced. Instead, we again found (at least in the context of the systems where, in addition to the free $CH_2CO_2^-$, the internal anhydride has been opened to form a disodium salt) a strong preference for the 7*S* configuration. Apparently, even in the "open" series, there are rigidifying influences—possibly arising from intramolecular hydrogen bonds—which favor the 7*S* diastereomers. Whether the preference for the 7*S* configuration extends to "open" systems that lack the array of lithium carboxylates, remains to be established. Such matters, as well as the biological properties of the newly fashioned and recognized 7*S* compounds, are the subjects of continuing investigation.

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[1] Representative synthetic approaches to **1** and **2**: K. C. Nicolaou, M. W. Harter, L. Boulton, B. Jandeleit, *Angew. Chem.* **1997**, *109*, 1243; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1194; K. C. Nicolaou, M. H. D. Postema, N. D. Miller, G. Yang, *Angew. Chem.* **1997**, *109*, 2922; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2821; H. M. L. Davies, R. Calvo, G. Ahmed, *Tetrahedron Lett.* **1997**, *38*, 1737; P. W. M. Sgarbi, D. L. J. Clive, *Chem. Commun.* **1997**, 2157; A. Armstrong, T. J. Critchley, A. A. Mortlock, *Synlett* **1998**, 552; N. Waizumi, T. Ito, T. Fukuyama, *Tetrahedron Lett.* **1998**, *39*, 6015; C. Chen, M. E. Layton, M. D. Shair, *J. Am. Chem. Soc.* **1998**, *120*, 10784; M. M. Bio, J. L. Leighton, *J. Am. Chem. Soc.* **1999**, *121*, 890; K. C. Nicolaou, P. S. Baran, R. Jautelat, Y. He, K. C. Fong, H.-S. Choi, W. H. Yoon, Y.-L. Zhong, *Angew. Chem.* **1999**, *111*, 532; *Angew. Chem. Int. Ed.* **1999**, *38*, 549.

[2] The first total syntheses of CP-225,917 and CP-263,114 were accomplished quite recently by Nicolaou and co-workers: a) K. C. Nicolaou, P. S. Baran, Y.-L. Zhong, H.-S. Choi, W. H. Yoon, Y. He, K. C. Fong, *Angew. Chem.* **1999**, *111*, 1774; *Angew. Chem. Int. Ed.* **1999**, *38*, 1669; b) K. C. Nicolaou, P. S. Baran, Y.-L. Zhong, K. C. Fong, Y. He, W. H. Yoon, H.-S. Choi, *Angew. Chem.* **1999**, *111*, 1781; *Angew. Chem. Int. Ed.* **1999**, *38*, 1676.

[3] a) O. Kwon, D.-S. Su, D. Meng, W. Deng, D. C. D'Amico, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 1978; *Angew. Chem. Int. Ed.* **1998**, *37*, 1877; b) O. Kwon, D.-S. Su, D. Meng, W. Deng, D. C. D'Amico, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 1981; *Angew. Chem. Int. Ed.* **1998**, *37*, 1880; c) D. Meng, S. J. Danishefsky, *Angew. Chem.* **1999**, *111*, 1582; *Angew. Chem. Int. Ed.* **1999**, *38*, 1485.

[4] Synthetic **3** is racemic. Furthermore, the absolute configuration of the natural products is not known. For purposes of discussion, we arbitrarily depict the absolute configuration as shown. The descriptors 7*R* or 7*S* is meaningful only in respect to the rest of the particular core structure enantiomer *drawn here*. We also note that in our earlier disclosure,[3c] this center (C7) had been numbered as C12 before the full construction of the CP system had been completed. We now return to the numbering system proposed by Kaneko et al.[5, 6]

[5] T. T. Dubrah, T. Kaneko, W. Massefski, Jr., E. B. Whipple, *J. Am. Chem. Soc.* **1997**, *119*, 1594.

[6] T. T. Dabrah, H. J. Harwood, Jr., L. H. Huang, N. D. Jankovich, T. Kaneko, J.-C. Li, S. Lindsey, P. M. Moshier, T. A. Subashi, M. Therrien, P. C. Watts, *J. Antibiot.* **1997**, *50*, 1.

[7] T. Okazoe, K. Takai, K. Utimoto, *J. Am. Chem. Soc.* **1987**, *109*, 951.

[8] The NOESY spectrum of **3** showed a cross peak between H7 and H17 instead of the expected H7 and H10. Every "closed" compound with the 7*S* configuration at C7 exhibited a quartet at $\delta = 4.1-4.2$ in their ^{1}H NMR spectra. By contrast, 7R-CP-263,114 (**2**) and its methyl ester (**10**) display a triplet at $\delta = 4.5-4.6$ in the ^{1}H NMR spectra.

  1433-7851/99/3821-3200 $ 17.50+.50/0

[9] Recent examples of singlet-oxygen oxidation of furans can be found in dysidiolide syntheses: a) E. J. Corey, B. E. Roberts, *J. Am. Chem. Soc.* **1997**, *119*, 12425; b) S. R. Magnuson, L. Sepp-Lorenzino, N. Rosen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1998**, *120*, 1615.

[10] The authors thank Dr. T. Kaneko and Dr. T. T. Dabrah of the Pfizer Central Research for providing a trace sample of natural CP-225,917 and several small samples of CP-263,114 from separate fermentation processes.

[11] Use of trimethylsilyldiazomethane leads to a trimethyl ester as the product from a ring opening of the anhydride. Some diazomethane methylations required 2-pentene as cosolvent to prevent side reactions at the two side-chain olefins.

[12] **9**: IR(film): $\tilde{\nu}$ = 2921, 1798, 1767, 1736 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.81 (s, 1H), 5.45–5.39 (m, 4H), 4.21 (dd, J = 12.2, 3.0 Hz, 1H), 3.71 (s, 3H), 3.29 (s, 1H), 3.25 (d, J = 17.5 Hz, 1H), 3.08 (d, J = 8.3 Hz, 1H), 2.95 (d, J = 17.5 Hz, 1H), 2.64 (dd, J = 19.2, 2.2 Hz, 1H), 2.29–2.20 (m, 3H), 2.04–2.00 (m, 3H), 1.94–1.89 (m, 3H), 1.64–1.62 (m, 6H), 1.25–1.14 (m); HR-MS (FAB) calcd for $C_{22}H_{38}O_9Na$ [M+Na]$^+$: 589.2413, found: 589.2391.

[13] **10**: IR(film): $\tilde{\nu}$ = 2927, 1792, 1768, 1740 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 5.66 (d, J = 1.9 Hz, 1H), 5.50–5.30 (m, 4H), 4.54 (t, J = 8.1 Hz, 1H), 3.73 (s, 3H), 3.53 (s, 1H), 3.25 (d, J = 17.4 Hz, 1H), 3.08 (d, J = 19.5 Hz, 1H), 2.93 (d, J = 17.4 Hz, 1H), 2.74–2.69 (m, 3H), 2.53 (m, 1H), 2.35–2.25 (m, 4H), 2.12 (dd, J = 13.6, 8.8 Hz, 1H), 1.94–1.91 (m, 2H), 1.64–1.62 (m, 6H), 1.25–1.14 (m); HR-MS (FAB) calcd for $C_{22}H_{38}O_9Na$ [M+Na]$^+$: 589.2413, found: 589.2415.

[14] The trace fermentation acid samples came from several different sources which differed in the amount of the 7*S* system **17** (and subsequently its methyl ester **11**). The ratio of 2:17 did not change following storage of the samples in our premises for five months at −78 °C.

[15] Separation conditions of **1**, **2**, **15**, and **17**: Reversed-phase HPLC column: Metachem Inertsil 5 μ ODS2, 0.002% H_3PO_4:CH_3CN = 4:6. Retention time: **15** (16 min), **1** (17 min), **2** (32 min), **17** (34 min). It is also crucial to inject the sample in a 1/1 mixture of 0.1% H_3PO_4 in CH_3CN. We note also that the chromatography per se does not effect the homogeneity of the samples. Hence, we are confident that the 7*S* isomer we detected was present in the original samples.

[16] Another pathway not invoking enediol **12** would involve a reversible C6-C7 α-ketol shift with an intervening rotation about the C6-C7 σ bond. This step would effectively epimerize C7 without the necessary scrambling of the ketol. For this "ketol-shift" pathway, as well as the enediol pathway, to be viable, it would be crucial that the 7-hydroxy-6-ketone be much more stable than the 6-hydroxy-7-ketone isomers in both the 7*R* and 7*S* series.

[17] Dess–Martin periodinane: a) D. B. Dess, J. C. Martin, *J. Org. Chem.* **1983**, *48*, 4155; b) D. B. Dess, J. C. Martin, *J. Am. Chem. Soc.* **1991**, *113*, 7277; c) S. D. Meyer, S. L. Schreiber, *J. Org. Chem.* **1994**, *59*, 7549; d) R. E. Ireland, L. Liu, *J. Org. Chem.* **1993**, *58*, 2899; review of TPAP/NMO oxidation: S. V. Ley, J. Norman, W. P. Griffith, S. P. Marsden, *Synthesis* **1994**, 639.

Electron Microscopy Reveals the Nucleation Mechanism of Zeolite Y from Precursor Colloids**

Svetlana Mintova, Norman H. Olson, and Thomas Bein*

Zeolites are crystalline, porous solids whose intricate pore and channel systems in the molecular size range of 0.3 to about 1.5 nm are the basis for their immense importance in catalysis, separations, and ion exchange.[1–4] Although numerous studies have addressed the preparation of zeolites, it has been very difficult to model the complex mechanism by which they assemble from framework constituent precursor species under hydrothermal synthesis conditions.

An improved understanding of the synthesis mechanism is pivotal for the design of new zeolites (only about 100 structures are known so far), and for the preparation of novel zeolitic assemblies such as zeolite thin films for membrane reactors, monoliths, or functional nanostructures.[5] Here we report direct, high-resolution electron microscopic evidence for the nucleation mechanism of zeolite Y (faujasite structure type; FAU) in nanoscale amorphous aluminosilicate gel particles, followed by full conversion of the gel aggregates into 25–35 nm large single crystals of zeolite Y. Further crystallization of the colloidal zeolite Y suspension is mediated by soluble aluminosilicate species.

Different mechanisms have been discussed regarding nucleation and crystallization of zeolites, based on experimental evidence obtained with various methods such as X-ray diffraction and scattering, solid-state NMR spectroscopy, atomic force microscopy, and electron microscopy.[6–22] These include transformation of the precursor gel phase, aggregation and realignment of preassembled building blocks containing template molecule/(alumino)silicate clusters, and assembly of soluble small species from solution. Most of the above techniques give information about the final crystalline product; however, imaging the initial stage of zeolite formation has not previously been possible.

Several molecular sieves, including zeolite A, Y, L, ZSM-5, silicalite-1, TS-1, and $AlPO_4$-5 can be made in colloidal form with particle sizes in the nanometer range.[23–28] Recently, we reported a detailed study of the very early stages of zeolite A

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